cell fate choices. More subtype-specific molecular markers will need to be identified, progresses in automatic image acquisition and in techniques to reliably identify cellular subtypes in clones and cell cultures will be required, and sophisticated mathematic modeling of cell fate choices based on a biased stochastic division will also be required. These advances will probably lead to an integral model combining both stochastic and deterministic inputs.

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The Clathrin Adaptor Complex Responsible for Somatodendritic Protein Sorting

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Neuronal proteins contain "address labels" that govern their localization. In this issue of *Neuron*, Farías et al. (2012) identify the machinery that recognizes one class of dendritic localization signals and establish its role in the polarization of dendritic proteins, including several postsynaptic receptors.

Nearly every aspect of neuronal function depends on the accurate trafficking of membrane proteins to specific sites within the axon or dendrites. While the complexity of protein targeting in neurons is extraordinary and neuronal dimensions are extreme, the basics of neuronal protein sorting are shared with many other polarized cells, such as epithelial cells. Many advances in understanding neuronal protein targeting have come from exploiting parallels between the two systems, a strategy first put forward by Dotti and Simons (1990).

In epithelia, the cytoplasmic domains of basolateral proteins contain short, linear motifs, including YxxΦ (where Φ is a bulky hydrophobic residue), and dileucine motifs, which direct their sorting. Near the end of the last millennium, parallel studies of neuronal proteins led to the first identification of dendritic sorting signals (Jareb and Banker, 1998; West et al., 1997). Based on work from many groups that have studied the localization of proteins in cultured neurons (reviewed by Horton and Ehlers, 2003; Lasiecka et al., 2009), as well as in transgenic animals (Mitsui et al., 2005), a clear picture has emerged: dendritic proteins contain sorting signals located within their cytoplasmic domains. Some of these signals resemble the YxxΦ motifs identified in basolateral proteins. Interestingly, dihydrophobic motifs that mediate basolateral sorting are not always sufficient for dendritic sorting (Silverman et al., 2005). What machinery recognizes these targeting signals to ensure that dendritic proteins are sorted into a distinct vesicle population? Many sorting events depend on clathrin adaptor proteins, which bind to and recruit cargo proteins to sites of vesicle budding. With the discovery that a novel form of the clathrin coat adaptor AP-1 (containing a distinct μ1B subunit) plays a critical role in basolateral sorting (Fölsch et al., 1999), the elucidation of the machinery for dendritic sorting seemed to be only a matter of time. This expectation turned out to be far too optimistic. It was soon established that AP-1B is not expressed in neurons, and, as the new decade dragged on, the machinery responsible for recognizing dendritic sorting signals remained as mysterious as ever. In this issue, Farías

et al. (2012) finally report progress on this key problem. They identify AP-1 as the missing link and demonstrate its essential role in the sorting of a variety of dendritic proteins, including several neurotransmitter receptors.

A recent collaboration between the Rodriguez-Boulan and Bonifacino laboratories showed that AP-1A (the form of AP-1 containing the μ1A subunit)—previously thought to be involved principally in trafficking between the *trans*-Golgi network, endosomes, and lysosomes—also plays a key role in the sorting of basolateral proteins (Gravotta et al., 2012). It appears that AP-1A works principally at the *trans*-Golgi complex while AP-1B acts during endosomal recycling. This result prompted the Bonifacino group to ask whether AP-1A might play a role in dendritic targeting in neurons (Farías et al., 2012). The authors first performed a rigorous mutational analysis to precisely identify the dendritic targeting signal in the transferrin receptor (TfR), a protein whose sorting has been well characterized in both MDCK cells and neurons. They identified a tyrosine-based YxxΦ motif in the cytosolic N-terminal tail of TfR that is essential for its dendritic polarity. Overexpressed wild-type TfR is about ten times more concentrated in the somatodendritic domain than in axons of cultured hippocampal neurons. Mutating the tyrosine residue at position 20 caused TfR to accumulate equally in both the axonal and somatodendritic domains.

The structural basis for binding between AP-1A and peptide sorting motifs has not been established, so the authors turned to the homologous AP-2 adaptor, which directs the clathrin-mediated endocytosis of proteins containing a YxxΦ motif (Kelly and Owen, 2011). Using the known crystal structure of the homologous μ2 subunit, Farías et al. identified residues on the C terminus of μ1A that are likely candidates for interacting with the N-terminal targeting signal of TfR. They then used a yeast two-hybrid screen to characterize the binding between μ1A and the TfR tail. Using this approach, they identified a tryptophan residue (W408) in μ1A that was essential for binding to the TfR tail. Interestingly, the coxsackievirus and adenovirus receptor (CAR), another dendritic protein whose sorting has been well characterized in epithelia, also interacts with μ1A, and this interaction is also disrupted by mutating W408.

Based on this structural analysis, the authors prepared mutant μ1A constructs that are unable to bind TfR and CAR and hence should act as dominant negatives when expressed in cultured neurons. Overexpression of this μ1A mutant (W408S) resulted in a complete loss of polarity for both TfR and CAR, fully comparable in magnitude to the results seen after mutating the sorting signals in these proteins. In addition to its role in cargo selection, AP-1 recruits clathrin to initiate vesicle budding. To demonstrate directly that dendritic sorting is clathrin dependent, the authors showed that expression of a dominant-negative construct that prevents clathrin assembly also disrupts the polarity of TfR.

Presumably, AP-1 complexes containing the mutant μ1A subunit were unable to sort TfR and CAR into dendritically targeted vesicles, allowing them to leak into axonal vesicles. To show this directly, the authors used live-cell imaging to follow the microtubule-based transport of TfR vesicles in living hippocampal neurons. Normally these vesicles undergo bidirectional transport in dendrites, but they only rarely enter the axon (Burack et al., 2000). Overexpression of μ1A-W408S resulted in a stream of TfR vesicles moving into the axon. These vesicles moved processively along the axon at high velocities, which is characteristic of vesicles that contain axonal proteins but is never observed for TfR vesicles. These data demonstrate that disruption of the interaction between the tail of TfR and the AP-1A adaptor resulted in the misincorporation of TfR into axonal carriers, most likely at the level of the *trans*-Golgi network in the neuronal soma.

Elegant as these experiments are from the cell biological perspective, there remains the question of whether AP-1A mediates the sorting of neuron-specific proteins crucial for dendritic signaling, such as neurotransmitter receptors. To address this question, the authors first conducted yeast two-hybrid analyses to assess possible interactions between μ1A and the cytoplasmic domains of several postsynaptic glutamate receptors. They established that the metabotropic glutamate receptor mGluR1 and the NMDA receptor subunits NR2A and NR2B bind μ1A and that the binding is disrupted by mutation of μ1A W408. The AMPA receptor subunits GluR1 and GluR2 do not bind μ1A. They then showed that overexpression of the W408S mutant resulted in a loss of polarity of GFP-tagged NMDA and metabotropic glutamate receptors, as well as endogenously expressed NMDA receptors (detected by immunofluorescence). Expressing the dominant-negative form of μ1A had no effect on the polarity of GFP-tagged or endogenously expressed AMPA receptors. These results show that AP-1A is essential for the sorting of several postsynaptic receptors and, quite possibly, for many other dendritic proteins as well.

Of course, such exciting results lead to further questions. First, what mechanisms are responsible for the sorting of dendritic proteins that do not bind to μ1A, such as AMPA receptors? Answering this question will also shed light on how many distinct dendritic vesicle populations there are and whether proteins destined for different dendritic subdomains, such as excitatory versus inhibitory synapses, travel in different vesicles. After dendritic proteins are sorted into a specific vesicle population, additional machinery must be recruited to ensure that these vesicles are transported only into dendrites and that they deliver their cargoes only at the correct sites. Two recent studies using novel experimental strategies have identified the kinesins and myosins that associate preferentially with TfR-containing vesicles (Al-Bassam et al., 2012; Jenkins et al., 2012). Could AP-1A play a role in recruiting such components to dendritic vesicles? Consistent with this idea, recent work shows that the kinesin KIF13A, a known binding partner of the β subunit of AP-1 (Nakagawa et al., 2000), is implicated in the transport of TfR vesicles (Jenkins et al., 2012). Finally, what regulates axonal protein sorting? The trafficking pathways that underlie axonal polarity remain the subject of active investigation, and no clear consensus has yet emerged concerning the nature or significance of sorting signals in axonally polarized proteins (Lasiecka et al., 2009). The strategy developed by Farías et al.—using a detailed analysis of the binding

between sorting motifs and adaptors to design reagents to manipulate sorting in living cells—could also be used to elucidate the machinery that directs axonal sorting.

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Dendritic Spikes Veto Inhibition

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How inhibition regulates dendritic excitability is critical to an understanding of the way neurons integrate the many thousands of synaptic inputs they receive. In this issue of *Neuron*, Müller et al. (2012) show that inhibition blocks the generation of weak dendritic spikes, leaving strong dendritic spikes intact.

Neurons come in two flavors: excitatory and inhibitory. Because excitatory neurons usually outnumber inhibitory neurons in most brain regions, it's not surprising that we know more about excitation than inhibition. This extends to our understanding of how inhibition regulates dendritic excitability. Although originally thought of as passive integrators of incoming synaptic inputs, we now know that dendrites express a range of voltage-gated channels and, as a result, can perform a variety of active forms of synaptic integration. This includes the generation of dendritic "spikes"—all-or-none, active responses initiated in localized dendritic regions or branches following the activation of dendritic voltage-gated sodium and/or calcium channels, as well as NMDA receptors, which derive their voltage dependence via external magnesium block. These active forms of dendritic integration have been studied in great detail over the last two decades, primarily due to advances that have allowed dendrites of neurons to be investigated directly using either electrophysiological or imaging techniques. What has been missing from the puzzle is an understanding of how this dendritic excitability is regulated by inhibition. In the current issue of *Neuron*, Müller and colleagues (2012) investigate the role of inhibition in regulating dendritic excitability in hippocampal CA1 pyramidal neurons. The authors focus on "recurrent" or "feedback" inhibition, evoked following antidromic activation of CA1 pyramidal neuron axons via stimulation of the alveus. Previous work indicates that stimulation of the alveus evokes at least two forms of recurrent inhibition, with a single stimulus recruiting primarily somatic and proximal dendritic inhibition, whereas brief trains (as used in the study by Müller and colleagues) also recruit a distal dendritic form of inhibition mediated by stratum oriens and lacunosum-moleculare (OL-M) cells (Pouille and Scanziani, 2004). The somatic and proximal dendritic inhibition evoked by alveus stimulation is likely to be mediated by a variety of interneuron subtypes, including axo-axonic cells, which target the axon initial segment, basket cells, which are primarily somatic, and bistratified cells, which target oblique and basal dendrites (Somogyi and Klausberger, 2005).

To generate dendritic spikes, the authors use local glutamate iontophoresis targeted to oblique and basal dendritic branches. Consistent with earlier work using glutamate uncaging (Losonczy et al., 2008), they find that glutamate iontophoresis generates localized dendritic spikes in a subset of basal and apical oblique branches of hippocampal CA1 pyramidal neurons. These local dendritic spikes can be detected at the soma as an abrupt change in the rate of rise of the somatic membrane potential,